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**ACTION OF ELECTROLYTES ON STIM-
ULATION OF THE SKELETAL
MUSCLE**

- I. THE INFLUENCE OF NEUTRAL SODIUM-SALT
SOLUTIONS ON CHEMICAL STIMULATION**
- II. THE COLD STIMULATION AND THE INFLUENCE
OF NEUTRAL SODIUM-SALT SOLUTIONS
ON THE COLD STIMULATION**

**A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

**DEPARTMENT OF PHYSIOLOGY
1934**

By IPING CHAO

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THE INFLUENCE OF NEUTRAL SODIUM-SALT SOLUTIONS ON CHEMICAL STIMULATION

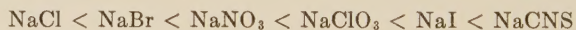
IPING CHAO

From the Department of Physiology, University of Chicago

Received for publication May 31, 1934

When an isolated frog's muscle is immersed in a pure isotonic Na-salt solution, the most obvious of all the changes that may occur in the muscle is the production of spontaneous rhythmic twitches. These twitches were first observed by Biedermann in 1879 in a curarized muscle placed in a 0.5 per cent NaCl solution made weakly alkaline by Na_2CO_3 , and were studied more thoroughly by Biedermann (1880), Ringer (1886), Loeb (1899) and many others. These twitches were also rightly ascribed by the early workers to the direct action of the salt solution on the muscle fibres, for they can be obtained in curarized as well as in uncurarized muscles.

The production of these spontaneous twitches suggests an increase in the irritability of the muscle. Thus, with particular reference to chemical stimulation, Zoethout (1904) mentioned an increase in irritability towards KCl in muscles placed in a NaBr or NaNO_3 solution. Lillie (1910) showed that the response of the skeletal muscle to various forms of chemical stimulation is increased by a preliminary immersion in a pure isotonic Na-salt solution, a process which may be designated for convenience as sensitization, and that the order of the relative sensitizing action for isotonic solutions of the following Na-salts is, in general, as follows:



In the present work a more extensive study of the conditions of the action of these Na-salt solutions on chemical stimulation will be presented.

Frog's sartorii were used throughout in these experiments. Experiments were performed in pairs, using a pair of sartorii from the same frog. Each muscle was attached to a light lever for graphic registration and was immersed in a beaker of about 250 cc. of Ringer's solution, which could be easily and rapidly replaced by another beaker of a sensitizing solution without disturbing the muscle. The procedure of experimentation has consisted in immersing a muscle in a sensitizing solution (e.g., a pure isotonic NaNO_3 solution) for a given period of time (the sensitization process); then this sensitizing solution was rapidly replaced by a stimulating solution (stimulation process). The stimulating solution contained 1 volume

of a M/8 KCl solution and 24 volumes of a M/8 NaCl solution (i.e., 5 millimols KCl per liter isotonic NaCl solution). This solution of the KCl and NaCl mixture usually produced a slight contracture in the muscle only, on transfer from Ringer's solution, and its strength of stimulation may be considered as threshold or slightly above. After a period of immersion in a pure isotonic Na-salt solution, the muscle responded immediately to the same stimulation with increased vigor, as shown by the increased height of the contracture (fig. 1). The increase in height before and after the sensitization, then, is a measure of the change in irritability



Fig. 1

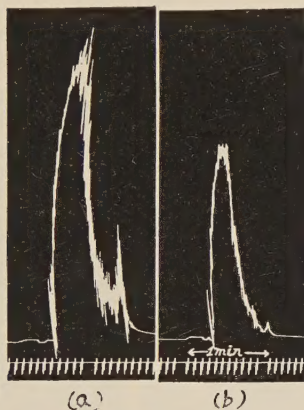


Fig. 2



Fig. 3

Fig. 1. An example of the increased response of the skeletal muscle to the stimulation of an isotonic NaCl solution containing 5 millimols KCl per liter, after the muscle was immersed in a pure isotonic NaNO_3 solution for 6 minutes. The response to the same stimulation before sensitization was negligible. In all the figures (read from left to right) the change of solutions was automatically marked by the rise and fall of the base line; a rise indicates the removal of a solution, and a fall indicates the application of another solution.

Fig. 2. The response of a curarized muscle to the chemical stimulation (a) after 10 seconds and (b) after 5 seconds in a pure isotonic NaCNS solution.

Fig. 3. Effect of changing from a pure isotonic NaCl solution at 25° to another at 0°C . Note the production of contracture in the latter.

to the particular chemical stimulation produced by the sensitizing solution under these conditions; and was employed throughout this work in the evaluation of the results obtained with different sensitizing solutions under different conditions.

The effect of anions on the sensitization. As shown by Lillie (1910) in the frog's gastrocnemius,¹ the relative effectiveness of different Na-salt solu-

¹ The author has confirmed Doctor Lillie's results on gastrocnemius with stimulating solution-containing KCl and NaCl.

tions in sensitizing the muscle to various forms of chemical stimulation follows the order of the Hofmeister series for anions. The same relationship is also true for the sartorius. The following experiment on a pair of sartorii from a curarized frog illustrates the influence of the anions very well. In this experiment each sartorius was sensitized in a pure isotonic Na-salt solution for two minutes before it was transferred into the stimulating solution. A period of at least 15 minutes was allowed for recovery in the Ringer's solution between two successive trials. The order of sensitization with a series of sensitizing solutions was reversed for the

TABLE 1

Percentage increase in response of a pair of curarized sartorii to chemical stimulation after a period of sensitization of two minutes in different pure isotonic Na-salt solutions

NaCl	NaBr	NaNO ₃	NaClO ₃	NaI	NaCNS
5	30	41	46	63	100

TABLE 2

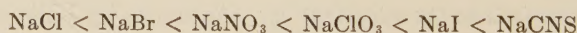
Percentage sensitization for different Na-salt solutions at different concentrations (diluted with Ringer's solution)

VOLUMES PER CENT PURE ISOTONIC Na-SALT SOLUTION	NaCl (2)*	NaBr (4)	NaNO ₃ (4)	NaI (4)	NaCNS (2)
100	100	100	100	100	100
80	50	74	73	110	106
60	0	27	22	98	108
50			16		
40		7	0	12	94
20				4	25
10					8
5					1.5

* The numbers in the brackets indicate the number of determinations.

two muscles to rule out any possible effect of fatigue or other changes in the tissue. The increase in height of response after the immersion in the NaCNS solution was taken as 100 per cent; the relative increases for the other Na-salt solutions are summarized in table 1.²

It is obvious from table 1 that NaCNS is the most effective sensitizing solution; the other solutions follow the order:



² Comparisons between any given two or three Na-salt solutions on many different pairs of sartorii gave the same relative position in the lyotropic series.

The effect of concentration of the Na-salt on the sensitization. The sensitization depends upon the concentration of the pure salt dissolved in the sensitizing solution. This is best shown by mixing pure isotonic Na-salt solutions with different amounts of Ringer's solution.³ In table 2 the increase in height of contracture in the stimulating solution after a period of sensitization in a given pure Na-salt solution for two minutes was taken as 100 per cent, and the responses after similar sensitizations in solutions of lower concentrations were calculated on the same percentage basis.⁴

When the values of the relative sensitizing action of the pure isotonic Na-salt solutions given in table 1 are substituted into table 2, the percentages of sensitization at other concentrations are recalculated and shown in table 3. Thus a composite graph can be constructed (fig. 6) to

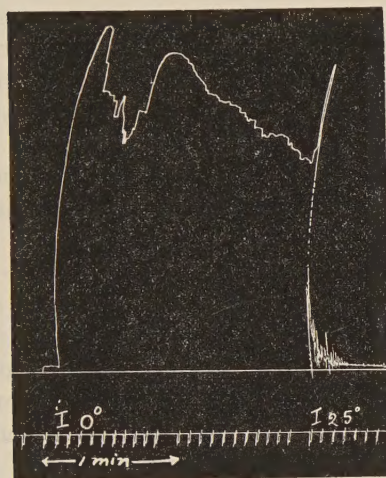


Fig. 4

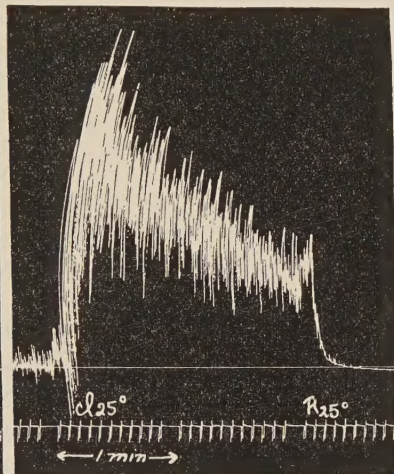


Fig. 5

Fig. 4. Effect of changing from a Ringer's solution at 25° to an isotonic NaI solution at 0°C. Note the immediate response of the muscle. Two minutes later, the cold NaI solution was replaced by another NaI solution at 25°C.

Fig. 5. Effect of changing from an isotonic NaCNS solution to an isotonic NaCl solution; both at 25°C.

³ Ringer's solution was used instead of some non-electrolyte solutions, ordinarily considered as indifferent, on account of the fact that these solutions (isotonic sucrose and dextrose solutions) were found to possess some undesirable action on the muscle by themselves.

⁴ NaClO₃ was left out of the series at this point on account of its anomalous behavior in the presence of NaCl. After a muscle is sensitized in a mixture of NaClO₃ and NaCl, or NaClO₃ and Ringer's solution, and an increased response to the chemical stimulation is obtained, subsequent sensitization in the same solution, or in a pure isotonic NaClO₃ solution, will no longer produce the same effect, although the muscle still responds to the action of the other Na-salt solutions.

show the relation between the concentration and the sensitizing action of different solutions.

Effect of duration of sensitization on the increase in response to chemical stimulation. The rapidity of the sensitizing action shows also a specific dependence on the nature of the anions. Thus in a pure NaCNS solution, a considerable increase in response is obtained after a period of sensitization of 10 seconds or less (fig. 2), and the maximal response is reached in less than half a minute. The durations of sensitization necessary to produce

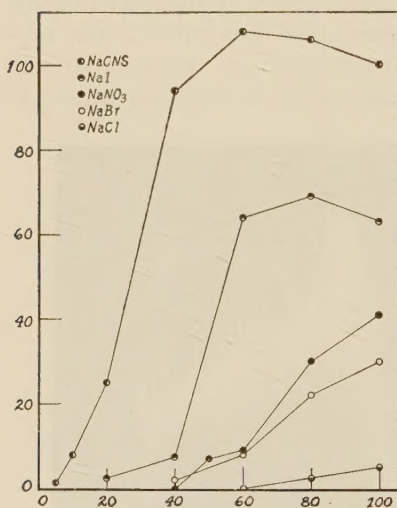


Fig. 6. A composite picture showing the relative sensitization effect and the influence of the concentration of the Na-salt solutions. Ordinates: percentage increase in response. Abscissae: percentage volumes of isotonic Na-salt solution in Ringer's solution.

again shown in the experiments on antagonism; this is summarized in table 5 (fig. 8).

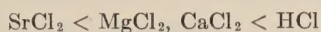
Similarly, the minimal concentrations of HCl, MgCl₂ and SrCl₂ necessary for complete suppression of the sensitizing action of several Na-salt solutions were determined, and the results are summarized in table 6.⁵

⁵ BaCl₂ differs from the other chlorides of the other alkali earth metals in that it possesses a powerful stimulating action, increasing the spontaneous twitches of muscle placed in pure Na-salt solutions (Ringer, 1886; Loeb, 1899). Thus even an addition of 0.01 millimol BaCl₂ to a liter of isotonic NaI solution produces much more vigorous twitches and considerable contracture and tends to diminish the response to the chemical stimulation. At higher concentrations of BaCl₂, these twitches and contracture obscure any subsequent response to chemical stimulation.

the maximal response for the other Na-salt solutions follow also the lyotropic order. Table 4 (fig. 7) gives the relative responses for different durations of sensitization in different solutions. The response after a period of sensitization of 10 minutes was considered as 100 per cent for NaCl; 6 minutes, for NaBr and NaNO₃; 2 minutes, for NaI; and 1 minute, for NaCNS. It should be mentioned, however, that the actual maximal response is not the same for all the Na-salt solutions.

Effect of CaCl₂, MgCl₂, SrCl₂ and HCl on the sensitizing action of the Na-salt solutions. The action of the pure Na-salt solutions in sensitizing a muscle to chemical stimulation is readily antagonized by the Ca-ion, and the concentration of Ca-salt required for this antagonism increases with the sensitizing effectiveness of the Na-salt. Hence the lyotropic order is

The order of the relative antagonistic action of these substances on sensitization indicated by these experiments is:



Effect of temperature on the sensitizing action of the Na-salt solutions. In general, low temperature tends to increase the stimulating action as well

TABLE 3

Relation between the concentration of the Na-salt solutions and their relative sensitizing action

VOLUMES PER CENT PURE ISOTONIC Na-SALT SOLUTION	PERCENTAGE INCREASE IN RESPONSE IN				
	NaCl	NaBr	NaNO ₃	NaI	NaCNS
100	5	30	41	63	100
80	2.5	22	30	69	106
60	0	8	9	64	108
50			7		
40		2	0	7.4	94
20				2.5	25
10					8
5					1.5

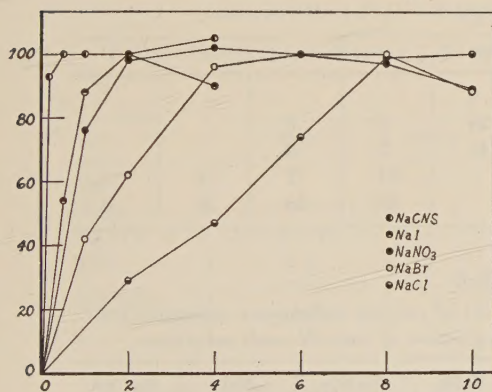


Fig. 7

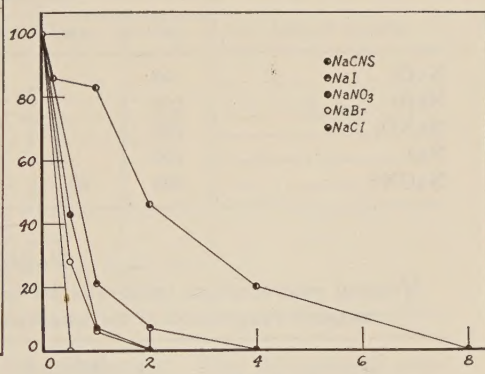


Fig. 8

Fig. 7. Effect of duration of sensitization in the Na-salt solutions. Ordinates: percentage increase in response. Abscissas: time of sensitization in minutes.

Fig. 8. Antagonistic action of CaCl_2 on sensitization. Ordinates: percentage increase in response. Abscissas: concentration of CaCl_2 in millimols per liter.

as the sensitizing action of the Na-salt solutions. Thus a Na-salt solution may produce more twitches in a muscle at room temperature, but a high contracture at low temperature, as illustrated by figures 3 and 4. The contracture produced in a cold Na-salt solution is not due to a sudden

change in temperature alone (although under certain conditions a sudden decrease in temperature may have a stimulating action by itself), for

TABLE 4

Percentage increase in response to chemical stimulation after immersions for various durations in different pure isotonic Na-salt solutions

	DURATION							
	10 seconds	30 seconds	1 minute	2 minutes	4 minutes	6 minutes	8 minutes	10 minutes
NaCl.....				29 (6)*	47 (4)	74 (7)	99 (2)	100
NaBr.....			42 (5)	62 (4)	96 (3)	100	100 (9)	88 (5)
NaNO ₃			76 (3)	98 (6)	102 (5)	100	97 (4)	89 (4)
NaI.....		54 (6)	88 (8)	100	90 (7)			
NaCNS.....	93 (7)	100 (6)	100	100 (6)	105 (4)			

* The numbers in the parentheses indicate the number of determinations.

TABLE 5

Percentage increase in response (100 per cent in pure Na-salt solutions) to chemical stimulation with addition of CaCl₂ to different Na-salt solutions (average of 2 determinations for each figure)

Duration of sensitization was 2 minutes

	CONCENTRATION OF CaCl ₂ (m.M. PER LITER)						
	0	0.2	0.5	1	2	4	8
NaCl.....	100		0				
NaBr.....	100		28	6	0		
NaNO ₃	100		43	7	0		
NaI.....	100			21	7	0	
NaCNS.....	100	86		83	46	20	0

TABLE 6

Minimal concentrations (millimols per liter) of various substances necessary for complete suppression of the sensitizing action of pure Na-salt solutions

	NaCl	NaBr	NaNO ₃	NaI	NaCNS
HCl.....		0.02	0.2		1*
CaCl ₂	0.5	1-2	1-2	2-4	8
MgCl ₂		4	4		8
SrCl ₂			8		16

* M/1000 HCl in an isotonic NaCNS solution produced contracture in the muscle (acid contracture).

considerable contracture occurs even on change from a Ringer's solution at 0°C. to a pure Na-salt solution at the same temperature.

The effect of temperature on the sensitization is illustrated in a series of experiments summarized in table 7.

In the above series of experiments both the sensitizing solutions and the stimulating solution were kept at the same given temperature. With the exception of NaCl, the response is, in general, greater at low temperature than at high temperature. In other words, there is a negative temperature coefficient for the sensitization to chemical stimulation.

TABLE 7

Percentage increase in response of muscle to chemical stimulation at different temperatures

Both the sensitizing solution and the stimulating solution were kept at the same given temperature. The response at 20° was taken as 100 per cent.

SENSITIZING SOLUTION	NUMBER OF DETERMINATIONS	DURATION OF SENSITIZATION IN MINUTES	TEMPERATURE OF SOLUTIONS			
			0°	10°	20°	30°
NaCl.....	4	6	0	44	100	74
NaBr.....	4	6	400	250	100	64
NaNO ₃	2	6	138	106	100	88
NaI.....	2	4	123	109	100	58
NaCNS.....	2	2	106	94	100	74

TABLE 8

Effect of exchange of anions on the spontaneous twitches of the skeletal muscle

FROM	TO				
	NaCl	NaBr	NaNO ₃	NaI	NaCNS
NaCl.....		--	--?	--	-----
NaBr.....	+++++?--		--	--?	--
NaNO ₃	+++++?--	--		--	-----
NaI.....	++	??	--		-----
NaCNS....	+++++++ +++++	++	+++++++ -	++++	

Analysis of the nature of the stimulating solution. The stimulating solution employed in these experiments contains 5 millimols KCl in a liter of an isotonic NaCl solution. The stimulation is really twofold. In the first place, the K-ion has a primary stimulating action followed by inhibition. Gellhorn (1928) has shown that the K-contracture is increased by the action of the Na-salt solutions in the same order as the Hofmeister anion series as described in this work. Secondly, in the case of Na-salts of anions other than chloride, there is a second effect which is apparently referable to an exchange of anions. This effect is illustrated in the experiments summarized in table 8. A sartorius was immersed in a given

pure Na-salt solution for four minutes at room temperature, and then transferred to a pure solution of another Na-salt at the same temperature. Changes in the amount and height of the spontaneous twitches are shown in table 8.

In table 8 each + sign indicates a single determination in which a definite increase in the amount and height of the spontaneous twitches, or even the production of considerable contracture, was the immediate result of change from one solution to another; a - sign, no increase; and a ? mark, a doubtful case. Thus, while change from NaCl to NaBr, NaNO₃, NaI and NaCNS did not increase the twitches, change from any of them to NaCl produced a definite and well-marked increase. No particular effect was seen on replacing any salt by NaCNS, yet change from NaCNS to any other salt of the series always produced vigorous twitches accompanied by contracture (fig. 5).⁶ The effect of the interchange of solutions between NaBr, NaNO₃ and NaI was not clearly defined.

DISCUSSION. The increase in response of the skeletal muscle to chemical stimulation under the conditions described above appears to be related to changes in the physical state of the structural colloids of the plasma membrane of the muscle fibres, as suggested by Lillie (1910). This assumption is justified in view of the following facts.

The order of the relative sensitizing action of the pure Na-salt solutions is that of the lyotropic series. This characteristic order of the relative influence of anions on the physical state of colloidal substances was first demonstrated by Hofmeister (1888) and his student (Lewith, 1888) on the precipitation of proteins. According to the colloidal theory of the action of ions in living tissues (Höber, 1926; Lillie, 1933), these anions have a dispersing action on certain structural colloids of the plasma membrane, and this change in some way facilitates the process of stimulation. The differential action of the anions may be referred to their differential adsorption by the colloidal substance. Thus Rona and Michaelis (1919) have shown that these anions are adsorbed in the same relative order by blood charcoal.

The rapidity with which the muscle responds to the change of its chemical environment indicates that the primary action of the ions is upon the surface of the muscle fibres. Immersion of a muscle in a pure Na-salt solution for one minute or less is sufficient to give rise to a well-marked increase in response. Furthermore, the response to subsequent chemical stimulation appears almost simultaneously with the change of solution. Such rapid response would exclude any possibility of diffusion, or penetra-

⁶ Incidentally the indirect CNS-contracture described by Gellhorn (1931) in the frog's sartorius on change from a mixture of Ringer's solution and isotonic NaCNS solution to a pure Ringer's solution is apparently due to a partial exchange of anions.

tion, as the primary cause in the process of stimulation; on the contrary, it suggests strongly some immediate action of the ions on the plasma membrane of the muscle fibres.

The negative temperature coefficient of the sensitization process (the response being greater at low temperature) seems again to rule out the possibility of diffusion as a primary factor in the change of irritability. On the other hand, processes that are characterized by a negative temperature coefficient, such as surface tension and its related phenomena (e.g., adsorption) are known to be important factors in relation to changes of the colloidal state. Finally Mond and Amson (1928) have shown by perfusion experiments that the resting uninjured muscle fibres are impermeable to anions. It is, therefore, reasonable to believe that the primary process involved in the action of these Na-salt solutions is an increase in the dispersion (or perhaps rather dispersibility) of certain colloidal components of the plasma membrane, the effect being referable to the adsorption of the anions. In some way this change facilitates the membrane alteration which appears to be the first stage in stimulation.

SUMMARY

When a frog's sartorius is immersed in a pure isotonic Na-salt solution for a short period, it becomes more responsive to the chemical stimulation of an isotonic NaCl solution containing 5 millimols KCl per liter.

The increase in irritability (sensitization) depends upon the nature of the anions and corresponds in degree to their relative positions in the lyotropic series; namely,



For any given Na-salt solution the increase in irritability is a direct function of its concentration. The minimal concentration of each salt necessary to produce a given increase depends, in general, upon its relative position in the lyotropic series.

The change of irritability in any pure Na-salt solution increases rapidly with the duration of immersion in that solution. The rapidity with which the maximal response is reached depends again upon the nature of the anions, and follows the lyotropic order.

The action of any given Na-salt solution is antagonized by HCl and chlorides of the alkali earth metals in the following order:



The minimal concentration of any of these antagonizing substances for each Na-salt solution depends again upon the nature of the anion of the Na-salt, increasing in the lyotropic order as above.

The skeletal muscle is more susceptible to chemical stimulation at low temperature, and the sensitizing action of all the Na-salt solutions (except NaCl) is greater at low temperature; i.e., exhibits a negative temperature coefficient.

It is suggested that these Na-salt solutions tend to facilitate or increase the dispersion of certain colloidal constituents of the plasma membrane, as a result of the differential adsorption of anions, and that the increase of irritability is a consequence. The correlation with the lyotropic order of the anions is thus explained.

The author is very much indebted to Dr. R. S. Lillie for suggesting this work and for his constant advice and kind guidance.

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THE COLD STIMULATION AND THE INFLUENCE OF NEUTRAL SODIUM-SALT SOLUTIONS ON THE COLD STIMULATION

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The production of heat rigor in skeletal muscle at high temperature is essentially an irreversible process, associated with a coagulative change of the muscle protoplasm. Reversible thermal contracture has been reported by Jensen (1909), produced by rapidly plunging a frog's sartorius through a hot Ringer's solution (or 0.6 per cent NaCl solution, or tap water, or mercury) at about 80°C. to a Ringer's solution near 0°C. This reversible thermal contracture can be obtained in curarized muscles as well as in muscles rendered non-irritable to electrical stimulation by narcosis or by previous freezing and thawing (Jensen, 1915). Jensen concluded that heat was the stimulus, but failed to appreciate the importance of the sudden large decrease in temperature.

Sommerkamp (1928) described a single observation on the production of contracture in the frog's ileo-fibularis by changing from a warm Ringer's solution at 30° to a cold one at 2° to 4°C. The present author has also had occasion to witness such contracture in a pair of apparently very irritable frog's sartorii on change from a Ringer's solution at 24° to a cold one at 0°C. These isolated observations suggest that under certain conditions cold may serve as an adequate stimulus. In fact, it has been observed that the irritability of the frog's muscle and nerve to electrical stimulation of relatively long duration is increased at low temperature (Gotch and Macdonald, 1896; Lucas and Mines, 1907). The irritability of the frog's sartorius to chemical stimulation is also increased at low temperature (Chao, 1934). In the present paper evidence will be presented to show that a sudden decrease in temperature can be made to stimulate the frog's skeletal muscle under suitable conditions and that the response of the skeletal muscle to this cold stimulation is influenced by the action of the neutral Na-salt solutions in the same way as its response to the chemical stimulation.

The procedure of experimentation is essentially the same as described in a previous paper (Chao, 1934). Experiments were performed in pairs, using in each case a pair of sartorii from the same frog. The stimulating effect accompanying a sudden decrease in temperature is made obvious in the same way that threshold, or subthreshold, chemical stimulation is

rendered effective (Lillie, 1910); namely, by increasing the irritability of the muscle by a temporary treatment with a pure isotonic Na-salt solution.

Thus, when a frog's sartorius is immersed in a pure isotonic Na-salt solution at room temperature for a short period, it becomes distinctly more sensitive to a sudden decrease in temperature. If the muscle is then suddenly transferred to a cold solution at $0^{\circ}\text{C}.$, it gives rise to a reversible contracture while in the cold solution (fig. 1). In the following experiment a pair of sartorii were immersed in an isotonic NaBr solution at 20° for 6

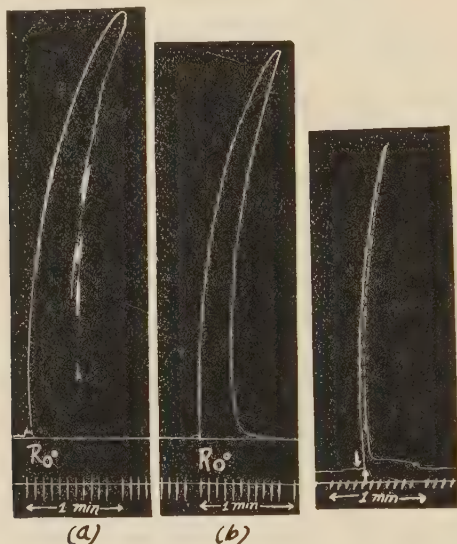


Fig. 1

Fig. 2

Fig. 1. Contracture in the cold Ringer's solution at $0^{\circ}\text{C}.$ after a period of immersion of the same muscle for 4 minutes, *a*, in a pure isotonic NaNO_3 solution at 20° , and *b*, in a pure isotonic NaBr solution at 20° . Note the contracture appears almost simultaneously with the change of solutions (or temperature), marked by a rise and fall of the base line in the tracings.

Fig. 2. Contracture in a Ringer's solution at 3° after a period of immersion in a pure isotonic NaCNS solution at 21° for 5 seconds.

minutes, then the NaBr solution was exchanged for various cold solutions at $0^{\circ}\text{C}.$ The results are shown in table 1.

The above experiments show conclusively that reversible contracture can be readily produced by a sudden decrease in temperature in muscles previously sensitized with a pure Na-salt solution. Even pure isotonic SrCl_2 or CaCl_2 solution can produce contracture under these conditions.¹

¹ The contracture produced in the cold isotonic CaCl_2 , or SrCl_2 , solution is lower than that in the cold Ringer's solution at the same temperature, indicating that CaCl_2 or SrCl_2 has a secondary effect in partly suppressing the full development of the cold contracture.

The contracture is not due to an exchange of anions, for under suitable conditions change from a pure isotonic NaCl solution at room temperature to a Ringer's solution at 0° has the same effect. Therefore the contracture must be a purely thermal reaction of the sensitized muscle to a sudden decrease in temperature.²

The contracture produced in a cold solution as a result of a sudden decrease in temperature is a function of the difference in temperature. The relation between the difference in temperature (Δt) and the height of contracture is clearly shown in table 2, which gives the results obtained on three pairs of sartorii. Each figure represents the average of two deter-

TABLE 1

Height of contracture produced in various cold solutions by a sudden decrease in temperature of 20 degrees after a period of immersion of a pair of sartorii in an isotonic NaBr solution at 20° for 6 minutes*

STIMULATING SOLUTION AT 0°C.	HEIGHT OF CONTRACTURE IN THE COLD SOLUTION IN MILLIMETERS (a) BEFORE AND (b) AFTER IMMERSION IN NaBr SOLUTION		INCREASE IN HEIGHT OF CONTRACTURE IN MILLIMETERS
	(a)	(b)	
Muscle A:			
Ringer's solution.....	0	100	100
M/4 dextrose solution.....	24	104	80
M/5 sucrose solution.....	50	109	59
M/10 SrCl ₂ solution.....	0	55	55
Ringer's solution.....	0	110	110
Muscle B:			
Ringer's solution.....	0	111	111
M/4 dextrose solution.....	40	121	81
M/4 sucrose solution.....	61	120	59
M/10 SrCl ₂ solution.....	0	69	69
Ringer's solution.....	0	119	119

* The height of contracture is obtained in all the experiments in this work from kymograph tracings recorded by levers of a magnification of about 7 times.

minations (one on each member of a pair of muscles). Each muscle was immersed in a pure isotonic NaBr solution for 6 minutes in every trial; from this solution it was transferred to a Ringer's solution at a different temperature.

In general, therefore, a sudden decrease in temperature under these conditions produces a contracture; a sudden increase in temperature, on the other hand, has no such effect. The least decrease in temperature necessary to produce a contracture (after a period of immersion in a pure isotonic

² Similar contracture can be also produced by sensitizing a curarized muscle to the cold stimulation.

NaBr solution for 6 minutes) is about 10 degrees; and the greater the decrease in temperature, the higher is the contracture.

The response to a given decrease in temperature (e.g., 10 degrees) at different temperatures, however, is not of the same magnitude. In the following experiments, muscles were immersed in pure isotonic NaNO_3 solutions at a given temperature (e.g., 30° , 20° , or 10°) for 2 minutes and then transferred to a Ringer's solution 10 degrees below the temperature of the NaNO_3 solution (i.e., 20° , 10° or 0° respectively). The average heights of contractures of 10 experiments are summarized in table 3.

TABLE 2
Relation between difference in temperature and height of contracture

TEMPERATURE OF NaBr SOLUTION	TEMPERATURE OF RINGER'S SOLUTION	Δt	HEIGHT OF CONTRACTURE IN MILLIMETERS
(1) 20°	22°	+2	0
20°	10°	-10	6
20°	5°	-15	17
20°	0°	-20	79
(2) 30°	0°	-30	98
20°	0°	-20	75
10°	0°	-10	60
0°	0°	0	0
(3) 2°	31°	+29	0
30°	25°	-5	0
30°	20°	-10	28
30°	10°	-20	57
30°	0°	-30	90

TABLE 3
Response of muscle to a decrease of temperature of 10 degrees after it has been immersed in a NaNO_3 solution at different temperatures (average of 10 experiments)

TEMPERATURE OF NaNO_3	TEMPERATURE OF RINGER	AVERAGE HEIGHT OF CONTRACTURE IN MILLIMETERS
30°	20°	14
20°	10°	8
10°	0°	62

It is obvious from table 3 that the response on change from 10° to 0° is by far the more pronounced, while the response on change from 30° to 20° seems to be a little higher than that from 20° to 10° .

The production of contracture in the cold Ringer's solution depends upon the duration of immersion in the Na-salt solution. The following experiment (table 4 and fig. 3) of immersing a pair of sartorii in a pure isotonic NaBr solution at 20°C . for varying lengths of time and then transferring them to a Ringer's solution at 0°C . shows that the height of re-

sponse to the cold stimulation increases rapidly with the duration of immersion and reaches a maximum after 6 minutes in the NaBr solution.

The action of the pure Na-salt solutions in sensitizing the skeletal muscle

TABLE 4

Effect of duration of immersion in a pure isotonic NaBr solution at 20° on the height of contracture produced on transfer to a Ringer's solution at 0°

DURATION OF IMMERSION IN MINUTES	HEIGHT OF CONTRACTURE IN MILLIMETERS		
	Muscle A	Muscle B	Average
1	22	56	39
2	39	53	46
4	45	64	55
6	50	65	58
8	44	71	58

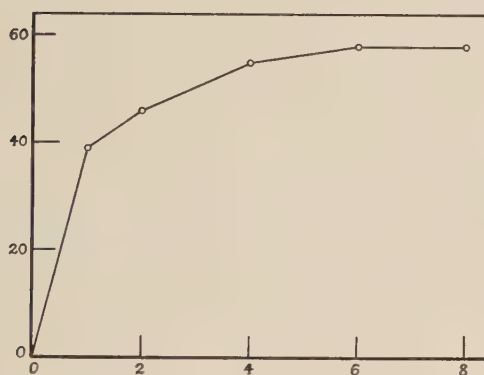


Fig. 3

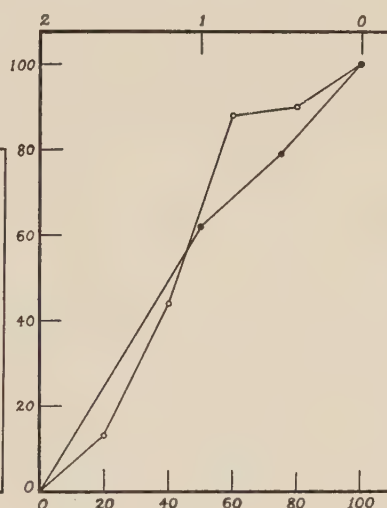


Fig. 4

Fig. 3. Relation between duration of immersion in a pure isotonic NaBr solution at 20° and the height of contracture in the cold Ringer's solution at 0°. Ordinates: height of contracture in millimeters. Abscissas: duration of immersion in minutes.

Fig. 4. *a*, Effect of concentration of NaI (circles), and *b*, effect of concentration of CaCl₂ (dots) on response to cold stimulation. Ordinates: percentage height of contracture. Abscissas: *a*, percentage volumes of isotonic NaI solution in Ringer's solution (lower), and *b*, millimols CaCl₂ per liter isotonic NaI solution (upper).

to the cold stimulation is rather rapid. Figure 2 illustrates the response of a muscle to a change of temperature of 18 degrees after a period of immersion in a pure isotonic NaCNS solution at 21° for 5 seconds only.

Considerable contracture appeared immediately on change to a Ringer's solution at 3°, and indeed the contracture approaches the shape of a single muscle twitch.

The response of the skeletal muscle to the cold stimulation, made possible by an immersion in a Na-salt solution, is a function of the concentration of the Na-salt used. Thus, in the following series of experiments a pair of sartorii were immersed in isotonic NaI solutions diluted with various amounts of a Ringer's solution at 20° for 2 minutes; the percentage heights of contractures in the Ringer's solution at 0° are given in table 5.

TABLE 5

Effect of concentration of NaI (diluted with Ringer's solution) on the response of muscle to the cold stimulation (Ringer's solution at 0°)

The response for the pure isotonic NaI solution was taken as 100 per cent.

VOLUMES PER CENT ISOTONIC NaI SOLUTION IN RINGER	PERCENTAGE HEIGHT OF CONTRACTURE		
	Muscle A	Muscle B	Average
100	100	100	100
80	93	87	90
60	93	83	88
40	41	47	44
20	21	5	13
0	0	0	0

TABLE 6

Effect of CaCl₂ on the action of NaI

The response for the pure NaI solution was taken as 100 per cent.

m. M. CaCl ₂ PER LITER ISOTONIC NaI SOLUTION	PERCENTAGE HEIGHTS OF CONTRACTURES IN RINGER'S SOLUTION AT 0°		
	Muscle A	Muscle B	Average
0	100	100	100
0.5	77	80	79
1	50	73	62
2	0	0	0

The action of a pure Na-salt solution in promoting contracture on transfer of a muscle to a cold Ringer's solution is, furthermore, antagonized by the addition of CaCl₂. Table 6 gives the percentage heights of contractures obtained in a pair of sartorii on change to a Ringer's solution at 0° after periods of immersions of 2 minutes in NaI solutions containing various concentrations of CaCl₂ at 20°.

The results in table 5 and table 6 are graphically represented in figure 4. The higher the concentration of NaI, the greater is the response to the cold stimulation. On the other hand, the higher the concentration of

CaCl_2 in the NaI solution, the less is the response; and the sensitizing action of NaI is completely suppressed when the concentration of CaCl_2 reaches 2 millimols per liter.

The contracture produced in the cold Ringer's solution after a period of immersion in a pure Na-salt solution also depends upon the nature of the anions of the Na-salts. The heights of contractures produced in the cold Ringer's solution at 0° after immersions of a pair of sartorii in different pure isotonic Na-salt solutions at 20° for periods of 4 minutes are given in table 7.

Thus the order of the relative effectiveness of these Na-salt solutions in increasing the response of the skeletal muscle to the cold stimulation is as follows:

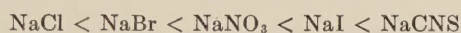


TABLE 7

Relation between the height of contracture and the nature of the anions of the Na-salt solutions

Na-SALT SOLUTION AT 20°C .	HEIGHT OF CONTRACTURE IN MILLIMETERS IN RINGER AT 0°C .		
	Muscle A	Muscle B	Average
NaCl*.....	0	0	0
NaBr.....	83	62	73
NaNO ₃	91	64	78
NaI.....	120	75	98
NaCNS.....	123	124	124

* Pure isotonic NaCl solution is also capable of producing contracture in the cold Ringer's solution; only in this particular experiment under these conditions it happened to be ineffective.

DISCUSSION. Two processes are involved in this cold stimulation: 1, a preliminary treatment with a pure Na-salt solution, and 2, a sudden decrease in temperature. The effect of the former treatment, preparatory in nature, is apparently to modify the colloidal structure of the plasma membrane of the muscle fibres (e.g., to increase the dispersion of some colloidal substance) and is antagonized by the addition of a sufficient amount of CaCl_2 . The effect of the latter treatment depends on the purely thermal phenomenon—simple decrease in temperature—and is relatively independent of the nature of the cold solution. Thus even pure isotonic CaCl_2 and SrCl_2 solutions are capable of producing a contracture under these conditions. The contracture appears immediately following the decrease in temperature and is perfectly reversible. The muscle relaxes almost completely in a short period while still in the cold solution. Therefore, whatever the stimulation process may be, the fundamental excitatory

changes in the cold stimulation are only temporary and similar in their general nature to those produced by chemical stimulation.

Furthermore, the influence of the Na-salt solutions on the cold stimulation shows, in general, a close parallelism to the influence on chemical stimulation (Chao, 1934). The lyotropic anion order of the Na-salts, the effect of duration of immersion in a Na-salt solution, the effect of concentration of a Na-salt, and the antagonistic action of CaCl_2 —all agree, both qualitatively and quantitatively, very well with the results obtained with the chemical stimulation. In short, whatever conclusions were drawn in reference to the action of the Na-salt solutions on chemical stimulation seem to apply equally well to cold stimulation.

SUMMARY

When a frog's sartorius is immersed in a pure isotonic Na-salt solution at room temperature for a short period of time, a reversible contracture appears on sudden change to a cold Ringer's solution, or any other cold solution. Evidence has been presented to show that this contracture is produced by the sudden decrease in temperature—the stimulus of cold. The height of contracture depends upon the difference in temperature on the one hand and upon the special action of the Na-salt solution on the other. The sensitizing or facilitating influence of the Na-salt solutions on the cold stimulation is essentially identical with that on the chemical stimulation.

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